

pHrodo™ BioParticles® Phagocytosis Kits for Flow Cytometry

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Revision A

Detailed protocol is available online at www.lifetechnologies.com/manuals.

BioParticles® Conjugates for Phagocytosis	Ex/Em (nm)*	Cat. no.†
pHrodo™ Red <i>E. coli</i> BioParticles® Conjugate	560/585	A10025
pHrodo™ Green <i>E. coli</i> BioParticles® Conjugate	509/533	P35381
pHrodo™ Green <i>S. aureus</i> BioParticles® Conjugate	509/533	P35382

* Conjugates are also compatible with 488-nm argon-ion laser excitation.

† Each kit also includes Lysis Buffer A, Buffer B, and Wash Buffer. Bring Lysis Buffer A and Buffer B to room temperature before use.

1. Collect whole blood samples in blood collection tubes containing heparin anticoagulant and place on ice for 10 minutes before use.
2. Resuspend pHrodo™ BioParticles® conjugates in 2.2 mL Buffer B and place on ice for 10 minutes before use.
3. Prepare two tubes each of positive controls, negative controls, and experimental samples as described in the detailed protocols.
4. Place one tube from each control and experimental sample on ice and the other tube in a 37°C water bath for 15 minutes.
5. Add 100 µL Lysis Buffer A to all tubes, vortex briefly, and incubate at room temperature for 5 minutes.
6. Add 1 mL Buffer B to all tubes, vortex briefly, and incubate at room temperature for 5 minutes.
7. Centrifuge at 350 × g for 5 minutes at room temperature. Discard the supernatant and resuspend the cell pellets in 1 mL Wash Buffer.
8. Repeat centrifugation and resuspend the cell pellets in 0.5 mL Wash Buffer for flow cytometry analysis.

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